

Drugs affecting coagulation factor synthesis

Citation for published version (APA):

Hemker, H. C. (1977). Drugs affecting coagulation factor synthesis. In D. Ogston, & B. Bennett (Eds.), *Haemostasis: Biochemistry, physiology, and pathology* (pp. 467-473). Wiley-Blackwell.

Document status and date:

Published: 01/01/1977

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

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CHAPTER TWENTY-THREE

Drugs Affecting Coagulation Factor Synthesis

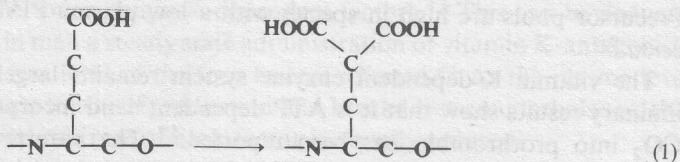
H. C. HEMKER

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INTRODUCTION

This chapter is mainly devoted to the description of the specific defects of the coagulation factors II, VII, IX and X that are caused by drugs with an anti-vitamin K action.

Vitamin K is necessary to bring about the final step in the biosynthesis of the coagulation factors II, VII, IX and X. The normal mechanism of protein synthesis in the liver produces individual precursor proteins for each of these factors. A vitamin K-dependent mechanism converts glutamic acid groups in these proteins to γ -carboxy glutamic acid residues. The proteins thus modified are the normal coagulation factors (1).



The dicarboxyl group functions to anchor the proteins *via* Ca^{2+} ions to a phospholipid surface in the process of coagulation. If the vitamin K-dependent mechanism is blocked, the precursors in certain species, including man, reach the circulation in the form of the so-called proteins induced by vitamin K absence or antagonists (PIVKAs).

The following subjects will be discussed in more detail:

- (1) The biosynthesis of the coagulation factors II, VII, IX and X
- (2) Pharmacological aspects of vitamin K antagonists
- (3) The role of PIVKAs in blood coagulation
- (4) Hormones and the level of clotting factors.

THE BIOSYNTHESIS OF THE COAGULATION FACTORS II, VII, IX AND X

After it has been shown that vitamin K is not a cofactor of prothrombin,^{1,2} the vitamin was thought to play a role in oxidative phosphorylation or to be a derepressor of RNA synthesis.³⁻⁵ These theories have been shown to be untenable.⁶⁻⁸ In addition, a role for vitamin K in peptide glycosylation and cellular secretion has been excluded.⁹ A hypothesis to explain the role of vitamin K in the synthesis of factors II, VII, IX and X, now generally accepted, was proposed on the basis of the observation that both the administration of vitamin K-antagonists and also vitamin K deficiency resulted in the appearance of a prothrombin-like protein in the plasma.¹⁰ From this it was inferred that vitamin K has no influence on polypeptide synthesis *per se*, but rather modifies a protein precursor so that it can function as a clotting factor. In the rat this mechanism has now been largely substantiated.⁹ Cycloheximide, a drug that blocks protein synthesis, does not prevent a rise of prothrombin level after administration of vitamin K to a deficient rat, but this prothrombin does not contain labelled amino acids administered together with the vitamin¹¹ indicating that *de novo* synthesis is adequately blocked.

It is possible to induce thrombin activity from the precursor by the use of a proteolytic enzyme from *Echis carinatus* venom. By this means it can be demonstrated that, in an animal in which protein synthesis is blocked, and which is deficient in vitamin K, a microsomal pool of precursor exists. Upon administration of vitamin K this is converted into a microsomal prothrombin pool within minutes. This material in the course of about an hour is transferred to the plasma.^{12,13} The microsomal precursor and the plasmatic PIVKA may or may not be identical;¹⁴⁻¹⁶ experimental evidence on this point is inconclusive. Precursor pools are high in species with a low plasma PIVKA level and *vice versa*.⁹

The vitamin K-dependent enzyme system remains largely undefined. Preliminary results show that it is ATP dependent⁹ and incorporation of labelled CO₂ into prothrombin has been reported.¹⁷ The nature of the vitamin K molecule suggests that it participates in an oxido-reduction reaction. Bell and Matschiner showed that vitamin K and vitamin K epoxide are equally active in the rat and the chicken, but in the warfarin-treated rat, labelled vitamin K accumulates in the epoxide form.¹⁸ It may well be that vitamin K-antagonists do not compete with vitamin K or a vitamin K derivative at the site where this substance functions in clotting factor synthesis, but inhibit a conversion of the

vitamin essential for its function. This would explain the observation that the pharmacokinetics of the vitamin and its antagonists are more complicated than could be explained by simple competition.^{19,20} Further studies by Bell and Matschiner²¹⁻²³ showed that the epoxide was not converted into vitamin K in the warfarin-treated rat and that the epoxide inhibits the action of vitamin K competitively if measured under conditions in which it cannot become converted into vitamin K. These workers postulate that the cycle $\text{vitamin K} \rightleftharpoons \text{vitamin K epoxide}$ catalysed by microsomal enzymes and inhibited by vitamin K analogues is an essential part of the physiological mechanism completing the formation of the coagulation factors.²¹⁻²³ Observations on congenitally warfarin-resistant rats appear to support this theory as in these animals the vitamin K oxidoreductase is insensitive to warfarin. Recent work by Sadovsky and Suttie²⁴ and Goodman *et al.*,²⁵ however, seem to exclude this possibility. Lowenthal suggested that coumarins act by blocking a specific cellular transport route for vitamin K.^{19,20,26-29}

The difference between a PIVKA and a coagulation factor in its final form has been well documented in the case of PIVKA-II and prothrombin.³⁰⁻³⁴ From this difference the effect of the vitamin K-dependent step on the precursor molecule can be inferred: in prothrombin up to ten glutamyl residues are converted into γ -COOH glutamyl residues. Such a chemical conversion may well be associated with an oxidation-reduction cycle. Yet the observation that CO_2 or HCO_3^- is necessary for this conversion^{35,36} leaves open the possibility that no oxidation-reduction is involved.

PIVKAs immunologically identical to factors IX and X have been demonstrated in the cow³⁷ and in man,³⁸ and a PIVKA-VII probably occurs in man.³⁹ It is likely that the vitamin K-dependent steps in the synthesis of the four vitamin K-dependent proteins are identical (i.e. the formation of γ -COOH glutamyl residues) although the proteins differ in primary structure.⁴⁰⁻⁴²

PHARMACOLOGICAL ASPECTS OF VITAMIN K ANTAGONISTS

The long term administration of a coumarin congener resulting in maintenance of a steady blood (c.q. hepatocellular) level causes an equal degree of inhibition of the synthesis of the four vitamin K-dependent factors.⁴³ This can be deduced from the fact that in man a steady state administration of vitamin K-antagonists causes an equally low level of the four factors affected.⁴⁴ As the plasma level reflects a steady state in which production and breakdown counterbalance, this means that the synthesis rate of each factor is equally reduced.

Coumarin in a dose that completely blocks synthesis causes the factors to disappear from the blood at different rates. This is because the factors have a different biological half-life time. Factor II disappears slowest ($t_{1/2} \sim 60$ h) followed by factor X ($t_{1/2} \sim 40$ h), factor IX ($t_{1/2} \sim 20$ h) and factor VII ($t_{1/2} \sim 7$ h). Dose variations, or variations in responsiveness resulting from the administration of other drugs, or from gastrointestinal disturbances, fever, *etc.*

will be most quickly reflected in the level of factor VII. This makes it mandatory that an overall test of the Quick type, when used to control anticoagulant therapy, is sensitive to factor VII. Some commercial thromboplastins do not meet this criterion.

Depending upon the amount of coumarin congener present, 5–25 mg vitamin K₁ will stimulate the synthesis of clotting factors. The effect starts 2–3 h after oral administration or 1½–2 h after intravenous injection. *Grosso modo* the re-appearance of the factors occurs at the normal rate of synthesis as calculated from the normal half-life times.^{45,46} Deviations from this rule can be explained by the combined action of the two complicating circumstances: firstly, an excess of precursor present in the hepatocytes can be converted quickly into normal coagulation factors; and secondly, when synthesis is switched on, the factors synthesized are distributed between both the intravascular and extravascular fluid.⁴⁷ A consequence of the accumulation of precursor has been observed by Lavergne and Josso.⁴⁸ At a low level of anticoagulation in the human a sufficient dose of vitamin K induces a temporary rise in the prothrombin level to above the 100% level.

Both synthesis rate and breakdown rate of coagulation factors are strongly influenced by the basal metabolic rate.⁴⁹ When metabolism is increased (fever, hyperthyroidism) both can be increased by a factor of two or more while in hypothyroidism both can be decreased several-fold.⁴⁵ It is interesting to note that synthesis and breakdown counterbalance each other, so that change in metabolic state does not influence the level of coagulation factors.

THE ROLE OF PIVKAs IN BLOOD COAGULATION

The fact that coumarin congeners (and vitamin K deficiency) cause PIVKAs to appear influences coagulation by several mechanisms. PIVKAs were discovered by the fact that one of them, presumably PIVKA-X in the human, causes inhibition of tests of the Quick time type. This is a complication to be considered in the control of anticoagulant therapy and can be used to diagnose vitamin K deficiency. On the other hand it has been demonstrated that PIVKA-II can yield normal thrombin, albeit at a slow rate, in the presence of normal prothrombinase (i.e. factor X_a, factor V, Ca²⁺ and phospholipid). Non-physiological prothrombin activators, such as staphylocoagulase or *Echis carinatus* venom, convert PIVKA-II into thrombin at approximately the same rate as they activate normal prothrombin. The level of factor II found under anticoagulation is therefore dependent upon the method of assay used. Immunological methods, staphylocoagulase, trypsin and *Echis carinatus* venom measure both normal prothrombin and PIVKA-II. Preliminary studies in this laboratory indicate that equimolar concentrations of prothrombin and PIVKA-II will not, however, cause equal effects in these three tests. Also the extent to which the tests react to a given concentration of PIVKA-II is dependent on the test used. The one-stage estimation using either congenitally factor II-deficient plasma or an artificial

reagent measures only normal prothrombin. The two-stage determination, due to the fact that normal prothrombinase slowly converts PIVKA-II into thrombin, coestimates PIVKA-II to a degree dependent upon the technique of the determination and the evaluation of the result.⁵⁰

The inhibition of thromboplastin times by PIVKA-X means that the plasma of an anticoagulated patient cannot be directly compared to a dilution of normal plasma. Yet dilutions of normal plasma are the only available substance to construct a reference curve in this type of test. Moreover, the degree of inhibition observed at a fixed inhibitor: factor ratio is dependent upon the dilution of the sample in the test system and upon the type of thromboplastin used. This has far reaching consequences for the standardization of the control of anticoagulant treatment. For a detailed discussion the reader is referred to the pertinent literature.⁵¹⁻⁵³

HORMONES AND THE LEVELS OF CLOTTING FACTORS

The administration of sex hormones is no longer used in the treatment of young haemophiliacs as no useful effect is obtained. Yet in some cases there appears to be an interrelationship between the level of factor IX and the hormonal status, for example, in haemophilia B_{Leiden}, the level of this factor is low in childhood (< 5%), but rises to 15% or more in puberty.^{52, 54} The mechanism underlying this change is still uncertain. Oral contraceptives reportedly cause a rise of factors X and VII or V, and VIII.⁵⁵⁻⁵⁸ The interpretation of these findings should be approached with care. On the one hand some authors do not find these rises (e.g. Lorrain and Harel⁵⁹), on the other, a mechanism has been described that may well explain spuriously high values of several clotting factors. Gjønnæss and Stormorken^{60, 61} have reported that in some plasmas upon storage at 4 °C factor VII is transformed into a more active form, probably *via* the kallikrein system. Not all plasmas show this activation; it is significantly more frequent in women than in men, and by far the most frequent in women taking oral contraceptives. We confirmed these findings and also found that the extent of activation depends upon the type of thromboplastin used. It is much more evident with the use of bovine thromboplastin than it is with human thromboplastin.⁶² It is therefore probable that the rise in clotting factor level observed by some authors is due to activation of factor VII rather than to a higher quantity of this or any other factor in the blood. It is also possible that the lowering of antithrombin III and other physiological proteinase inhibitors under the influence of sex hormones observed by some authors^{63, 64} reflects increased consumption of these proteins due to the presence of a high level of active proteinases.

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